Associations of dietary calcium intake and calcium supplementation with myocardial infarction and stroke risk and overall cardiovascular mortality in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition study (EPIC-Heidelberg)

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Abstract: BACKGROUND: It has been suggested that a higher calcium intake might favourably modify cardiovascular risk factors. However, findings of an ultimately decreased risk of cardiovascular disease (CVD) are limited. Instead, recent evidence warns that taking calcium supplements might increase myocardial infarction (MI) risk. OBJECTIVE: To prospectively evaluate the associations of dietary calcium intake and calcium supplementation with MI and stroke risk and overall CVD mortality. METHODS: Data from 23 980 Heidelberg cohort participants of the European Prospective Investigation into Cancer and Nutrition study, aged 35-64 years and free of major CVD events at recruitment, were analysed. Multivariate Cox regression models were used to estimate HRs and 95% CIs. RESULTS: After an average follow-up time of 11 years, 354 MI and 260 stroke cases and 267 CVD deaths were documented. Compared with the lowest quartile, the third quartile of total dietary and dairy calcium intake had a significantly reduced MI risk, with a HR of 0.69 (95% CI 0.50 to 0.94) and 0.68 (95% CI 0.50 to 0.93), respectively. Associations for stroke risk and CVD mortality were overall null. In comparison with non-users of any supplements, users of calcium supplements had a statistically significantly increased MI risk (HR=1.86; 95% CI 1.17 to 2.96), which was more pronounced for calcium supplement only users (HR=2.39; 95% CI 1.12 to 5.12). CONCLUSIONS: Increasing calcium intake from diet might not confer significant cardiovascular benefits, while calcium supplements, which might raise MI risk, should be taken with caution.

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ABSTRACT (248 words)

Background: It has been suggested that a higher calcium intake might favorably modify cardiovascular risk factors. However, findings of an ultimately decreased risk of cardiovascular disease (CVD) are limited. Instead, recent evidence warns that taking calcium supplements might increase myocardial infarction (MI) risk. The objective of this study was to prospectively evaluate the associations of dietary calcium intake and calcium supplementation with MI and stroke risk and overall CVD mortality.

Methods: Data from 23,980 Heidelberg cohort participants of the European Prospective Investigation into Cancer and Nutrition study, aged 35-64 years and free of major CVD events at recruitment, were analyzed. Multivariate Cox regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: After an average follow-up time of 11 years, 354 MI and 260 stroke cases and 267 CVD deaths were documented. Compared with the lowest quartile, the third quartile of total dietary and dairy calcium intake had a significantly reduced MI risk, with a HR of 0.69 (95% CI: 0.50, 0.94) and 0.68 (95% CI: 0.50-0.93), respectively. Associations for stroke risk and CVD mortality were overall null. In comparison to non-users of any supplements, users of calcium supplements had a statistically significantly increased MI risk (HR: 1.86; 95% CI: 1.17, 2.96), which was more pronounced for calcium supplement only users (HR: 2.39; 95% CI: 1.12, 5.12).

Conclusions: Increasing calcium intake from diet might not confer significant cardiovascular benefits, while calcium supplements, which might raise MI risk, should be taken with caution.

INTRODUCTION

Epidemiological studies have consistently reported inverse associations between dietary calcium intake and the risk of hypertension, obesity, and type 2 diabetes,[1-8] suggesting that a reasonably higher intake of this mineral might ultimately decrease the occurrence of cardiovascular events. Such a plausible health benefit has indeed been observed by several epidemiological studies. In three prospective studies, dietary calcium intake was significantly inversely associated with the ischaemic stroke risk.[9-11] A fourth study also found a statistically significant inverse association between dietary calcium intake and the mortality from ischaemic heart disease.[12] In a Swedish male cohort, the association between dietary calcium intake and overall CVD mortality was inverse and of borderline statistical significance.[13] However, except for these supportive findings, the majority of observational studies reported null associations.[14-21]

Calcium supplements, which are commonly recommended to elderly people, particularly postmenopausal women, to maintain their bone health, have also been suggested as beneficial agents to improve serum cholesterol profile [22-24] and to control hypertension.[25] However, no strong epidemiological evidence suggests that calcium supplementation might provide cardiovascular benefits.[10, 12, 14, 16] Instead, two recent meta-analyses of clinical trials have prompted a warning that calcium supplements might increase an individual's risk of having myocardial infarction (MI).[26, 27]

In the present study, we aimed to prospectively examine the associations of dietary calcium intake, in total or separated as from dairy sources and from non-dairy sources, and calcium supplementation with MI and stroke risk and overall CVD mortality in a German cohort.

METHODS

Study population

Heidelberg cohort is one of the two German cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. In 1994-1998, the EPIC-Heidelberg cohort recruited 25,540 local residents, who were then aged 35-64 years. A detailed description of the recruitment procedures has been published elsewhere.[28] The ethics committee of the Heidelberg University Medical School approved the study protocol and all participants provided an informed consent. In this study, we excluded participants who had a diagnosis of MI, stroke, or transient ischaemic attack at baseline (n=1,322). We also excluded participants whose daily energy intake fell into the top or the bottom 0.5 percentile (men: <887/> 5,582 kcal/day; women: < 70 / > 4,381 kcal/day; n=257), and eventually had 23,980 participants remained for analysis.

Assessment of dietary calcium intake and calcium supplementation

A self-administered food frequency questionnaire (FFQ), which had been validated using 12 24-hour dietary recalls in a subsample of 104 participants from the two German cohorts,[29, 30] was used to assess consumption of 148 food items in the 12 months before the date of recruitment. Dietary calcium intake was derived using the German Nutrient Database BSL, version II.3. Within the entire EPIC-Germany, dairy foods and non-alcoholic beverages were the main sources of dietary calcium, providing 39.9% and 28.2% of the daily intake, respectively.[31] The

Spearman correlation coefficient between the FFQ and the 12 24-hour dietary recalls was 0.58 for dairy foods and 0.70 for non-alcoholic beverages.[30]

In a baseline interview and follow-up questionnaire surveys, participants were asked if they had regularly taken vitamin/mineral supplements in the past 4 weeks, where "regularly" was defined as daily use for at least 1 week or non-daily use of at least 5 doses on a regular basis. Self-reported supplements were coded using the Anatomical Therapeutic Chemical (ATC) classification system. Data on dosage were not collected. In this study, we separated cohort participants into users of calcium supplements (ATC code A12A), users of other supplements (containing users of unspecified supplements), and non-users of any supplements. Users of calcium supplements were also separated into calcium supplement only users and users of calcium supplements plus other vitamins/minerals.

Ascertainment of cardiovascular outcomes

Incident cardiovascular events during follow-up were reported by participants or their next of kin in follow-up surveys. Reported cardiovascular events were verified by tracking medical records or official death certificates. In the present study, cardiovascular events of interest, which were coded using the International Classification of Diseases 10th version (ICD-10), were incident MI (ICD-10 codes I21-I23), incident stroke (ICD-10 codes I60-I69), and overall CVD mortality (ICD-10 codes I00-I99).

Statistical analysis

Dietary intakes of calcium and other nutrients were adjusted for total energy intake of 2,200 kcal/day for men and 1,700 kcal/day for women using the residual method.[32] Residuals were obtained from sex-specific linear regressions of the log-transformed calcium intake on the log-transformed total energy intake. The energy-adjusted calcium intakes were categorized into quartiles using sex-specific cut-off points. The lowest quartiles were used as reference groups.

Age- and sex-adjusted baseline characteristics of participants across quartiles of energy-adjusted total dietary calcium intake and across calcium supplementation status were compared using analysis of covariance for continuous variables and logistic regression for binary variables.[33] Multivariate Cox regressions were performed to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). To avoid violation of the proportionality assumption, all models were stratified by the rounded age at recruitment (1-year category). The following potential confounders were adjusted for in the analyses of dietary calcium intake: sex, age at recruitment, the highest educational level (no/primary school, secondary/technical school, and university), physical activity (inactive, moderately inactive, moderately active, and active), body mass index (BMI, kg/m^2), smoking categories (never smoker; former smoker, quit ≥10 years and quit <10 years; current smoker, ≤10, 11-20, and >20 cigarettes/day), lifetime alcohol intake (g/day), total energy intake (kcal/day), energy-adjusted dietary intakes of vitamin D (μg/day), saturated fatty acids (g/day), and total protein (g/day), self-reported diabetes mellitus at recruitment, and use of calcium supplements. Other baseline characteristics, including dietary intakes of fiber, vitamin C, and folic acid, self-reported hypertension, hyperlipidemia, and regular use of antihypertensive drugs, lipid-lowering drugs, and non-steroidal anti-inflammatory drugs (NSAIDS) only slightly affected the HRs, and therefore they were not included into the final models. Dairy and non-

dairy calcium intakes were mutually adjusted for when they were analyzed separately. Linear trends were examined using the likelihood ratio test, in which the median intakes of quartiles were modeled as a continuous variable.

Multivariate Cox models that examined the effects of calcium supplementation also adjusted for total dietary calcium intake, self-reported hyperlipidemia, and use of NSAIDs, as they appreciably affected the risk estimates. In addition to examining the effect of calcium supplementation at baseline, extended Cox regression models [34] were fitted to examine the effects of the most recent and the cumulative calcium supplementation by using the data on supplement use collected during the follow-up. All analyses were repeated after exclusion of CVD events that occurred in the first 2 years of follow-up.

All statistical tests were two-sided, with P <0.05 being considered statistically significant. SAS software (version 9.2; SAS Institute, Cary, NC) was used to perform all statistical analyses.

RESULTS

Table 1 shows the baseline characteristics of participants by quartiles of the energy-adjusted total dietary calcium intake and by calcium supplementation status. A higher dietary calcium intake was mainly associated with favorable factors, including younger age, higher likelihood of having a university degree and being physically active, less likelihood of being overweight/obese (BMI ≥ 25 kg/m^2) and current smokers, and an averagely shorter smoking duration and lower lifestyle alcohol consumption. Dietary calcium intake was also positively associated with dietary vitamin D and saturated fatty acid intake and the likelihood of taking calcium supplements. Compared

with non-users, users of calcium supplements were more likely to be women, physically more active, and less likely to be overweight/obese. On the other hand, users of calcium supplements had an older age, an overall lower educational level, and a longer duration of smoking.

After an average follow-up time of 11 years, 354 MI cases, 260 stroke cases, and 267 CVD deaths were documented. After adjustment for potential confounders, a statistically significant inverse association was only observed between total dietary calcium intake and MI risk for the third quartile compared with the lowest quartile (HR: 0.69; 95% CI: 0.50, 0.94; Table 2). Compared with the lowest quartile, the second quartile of total calcium intake had a statistically significantly increased stroke risk (1.50; 95% CI: 1.06, 2.11), which became non-significant after exclusion of the first 2 years of follow-up. For source-specific calcium intake, the previously observed reduction of MI risk in the third quartile of total calcium intake remained in the third quartile of dairy calcium intake (HR: 0.68; 95% CI: 0.50, 0.93; Table 3). None of the linear trend tests was statistically significant. Further exclusion of supplement users from analyses did not substantially change the risk estimates for total and source-specific dietary calcium intakes.

As shown in Table 4, users of calcium supplements had a statistically significantly increased MI risk when compared with non-users of any supplements (HR: 1.86; 95% CI: 1.17, 2.96). This association was more pronounced for calcium supplement only users (HR: 2.39; 95% CI: 1.12, 5.12) and persisted after MI cases that occurred in the first 2 years of follow-up were excluded (HR: 2.70; 95% CI: 1.26, 5.79). As shown in the extended Cox regression models, the most recent but not the cumulative calcium only supplementation was significantly inversely

associated with MI risk (HR: 2.17; 95% CI: 1.06, 4.42). No statistically significant association was observed between calcium supplementation and either stroke risk or overall CVD mortality.

DISCUSSION

In this prospective cohort study, total, dairy, or non-dairy calcium intake did not manifest an overall statistically significant inverse association with cardiovascular risk, except for a likely reduction of MI risk associated with a moderately higher dairy calcium intake. However, this study also suggests that MI risk might be substantially increased by taking calcium supplements.

The association between dietary calcium intake and MI risk has been rarely reported. In our cohort, a moderately higher dietary calcium intake (the third quartile, mean=820 mg/day) was statistically significantly associated with a 30% lower MI risk. However, this inverse association became non-significant for men (HR: 0.80; 95% CI: 0.56, 1.14) but more significant for women (HR: 0.43; 95% CI: 0.22, 0.82). A possible explanation to this gender-related disparity might be, if we assume the observed inverse association were false, that a higher dietary calcium intake was associated with certain unadjusted confounders in women but not in men. In a cohort study of middle-aged and elderly US male health professionals, which has perhaps been the only large-scale study reporting this association so far, no significant results were found, even in participants who had similar calcium intakes for which the association was statistically significant in the present study.[14] In addition to inclusion of only men, the US study used the standard method, whereas we used the residual method, to adjust for total energy intake. It has been noted that these two methods might generate substantially different results if nutrient variables were categorized into quantiles.[35] In our cohort, the inverse association was only

confined to dairy calcium intake, suggesting a possibility that the inverse association might be caused by other unknown nutrients coexisting in milk products. For instance, three case-control studies have reported an inverse association between dairy fat biomarkers (pentadecanoic and heptadecanoic acid) and the risk of a first-ever MI or cardiovascular risk factors.[36-38]

Our finding of an overall null association between total, dairy, or non-dairy calcium intake and stroke risk consists with the majority of previous findings,[15-17, 21] although two studies of Japanese/Japanese immigrants and one study of US women observed a statistically significant inverse association.[9, 10, 39] An easy interpretation to this inverse association is that the potential anti-hypertensive effect of calcium might cause an ultimate reduction in ischemic stroke risk. However, none of these three studies reported a similar reduced risk of hemorrhagic stroke, which is equally likely to be caused by hypertension. In addition, the much lower dietary calcium intake in two Japanese studies limits its comparability with results from a western population. A meta-analysis found that a higher consumption of milk, a major dietary source of calcium in western countries, was statistically significantly associated with a decreased risk of ischaemic stroke.[40] However, it is hard to conclude that this inverse association is mainly driven by the calcium content.

In the present study, no overall statistically significant association was observed between total, dairy, or non-dairy calcium intake and CVD mortality. This finding is in line with two other studies.[18, 20] In a third study of Swedish men, the association was almost statistically significant (HR highest versus lowest tertile: 0.77; 95% CI: 0.58, 1.01; P_{trend}=0.06).[13] In comparison to the studies reporting null associations, including the present study, the Swedish

study had a significantly higher dietary calcium intake (overall mean=1,400 mg/day; mean for the highest tertile=1953 mg/day). The effect of dietary calcium intake at such a high level on CVD mortality, however, might not be possible for many observational studies to investigate.

We observed a statistically significantly increased MI risk among users of calcium supplements, in agreement with the results of two meta-analyses of clinical trials.[26, 27] The more pronounced increase was observed among calcium supplement only users, suggesting that this adverse effect is mainly from calcium supplements themselves. Unlike the baseline and the most recent calcium supplementation, the model for cumulative calcium supplementation only yielded a statistically non-significant inverse association with MI risk. However, it should be noted that this model was based on a hypothetical linear relationship between times of self-reported calcium supplementation and MI risk. Therefore, we conclude that for an elderly population, this adverse effect might not rely much on a very long term of supplementation. So far, only very few observational studies have reported on the association between calcium supplementation and MI risk. In the US study of male health professionals, this association was null.[14] In a small British cohort study of women, calcium plus vitamin D supplementation was also not associated with MI risk.[41] There has been one study that observed an increased risk of coronary heart disease among women who took calcium or calcium plus vitamin D supplements.[42] To the best of our knowledge, the present study is the first observational study to report a possible adverse effect of calcium supplements on MI risk. With respect to the associations between calcium supplementation and stroke risk and overall CVD mortality, the present study and all previous observational studies are in agreement on the null associations.[10, 12, 14, 16]

The underlying mechanisms of the adverse effect of calcium supplements on MI risk might be in relation to the acute increase in serum calcium, which has been observed after ingestion of calcium supplements, but not after eating calcium-rich foods.[43, 44] Several studies have observed a positive association between serum calcium levels and vascular calcification.[45, 46] Too much calcium in serum might cause this pathological change by influencing calcification modulators such as pyrophosphate and binding to the calcium sensing receptors on vascular smooth muscle cells.[47] Other studies have reported positive associations of serum calcium levels with some predictive biomarkers of CVD, such as fasting insulin and lipid measures,[48, 49] and more directly, with MI risk.[50-52] As a key regulator of calcium metabolism, parathyroid hormone has also shown a close relationship with cardiovascular risk.[53-55] Therefore, in order to achieve a better understanding of the mechanisms behind the adverse cardiovascular effect of calcium supplementation, its interactions with of parathyroid hormone should be investigated.

The present study has several strengths, such as its prospective design, relatively large sample size, and an average follow-up time of more than 10 years. However, the present study also has several important limitations. Firstly, our dietary data suffered from measurement errors, which are unavoidable in application of a FFQ. In addition, one single measure of dietary nutrient intakes at baseline apparently could not capture the long-term variation, as we know that individuals might modify their diet after onset of certain diseases or enhancement of health consciousness. The same problem also stands for those modifiable lifestyle confounders. Secondly, the present study only excluded pre-existing MI, stroke, and transient ischaemic attack. Failing to exclude individuals with other pre-existing CVD subtypes might attenuate an

inverse association with CVD mortality if they were more likely to have a calcium-rich diet than relatively healthy individuals, or the other way round. However, the influence of this incomplete exclusion should be minor, as MI and stroke together account for the vast majority of CVD. There is also no evidence suggesting that individuals with pre-MI conditions are more likely to take calcium supplements. On the contrary, the positive association between calcium supplementation and MI risk was strengthened by exclusion of the first 2-year follow-up. Thirdly, in most of the clinical trials included in the above-mentioned meta-analyses,[26, 27] calcium in elemental form was administered at 1,000 mg/day or higher. Therefore, whether a lower dosage will still pose an extra MI risk should be examined. Unfortunately, the present study could not answer this question due to lack of detailed data. Lastly, it needs to be noted that 44.5% of vitamin/mineral users in the present study did not report the names of their supplements, and we therefore only identified a limited number of calcium supplement users, who accounted for 3.6% of all cohort participants. This prevalence is lower than that observed in a small German elderly population (about 8% in men and 27% in women).[56] It is also lower than the prevalence (11.0%) reported by a US national survey.[57] It is possible that the unreported calcium supplementation would affect the accuracy of our results if identified calcium supplement users were different from those unidentified ones in cardiovascular risk profile.

In conclusion, the present study suggests that increasing dietary calcium intake from diet might not confer significant cardiovascular benefits, while calcium supplements, which might raise MI risk, should be taken with caution.

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Competing interests: None

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Table 1. Age- and sex-adjusted baseline characteristics of participants by sex-specific quartile of dietary calcium intake and calcium supplementation status, the EPIC-Heidelberg cohort, 1994 -1998

	Total dietary calcium intake in quartiles [1]				Calcium supplementation		
	1 (low)	2	3	4 (high)	Calcium supplements	Other supplements	Non-use of any supplements
No. of participants [2]	5,986	5,993	5,998	6,003	851	7,170	15,959
Total dietary calcium intake [1] (mg/day)	513	675	820	1130†	828	820	766†
Dairy calcium intake [1] (mg/day)	188	330	466	780†	477	470	426†
Non-dairy calcium intake [1] (mg/day)	324	345	353	351†	352	349	340†
Dietary vitamin D intake [1] (µg/day)	3.0	3.2	3.3	3.4†	3.4	3.4	3.2†
Dietary intake of saturated fatty acids [1] (g/day)	30.0	30.8	31.3	32.3†	30.6	30.7	31.3†
Dietary intake of total protein [1] (g/day)	65.2	67.3	69.5	74.5†	70.3	69.2	69.0
Smoking duration (years)	13.5	11.8	11.5	11.7†	13.0	12.3	12.0†
Lifetime alcohol intake (g/day)	21.3	17.7	15.2	13.8†	17.3	17.1	17.0
Age at recruitment (years)	51.2	50.6	50.3	50.5†	53.6	51.8	50.0†
Women (%)	54.1	54.0	54.2	54.0	72.4	60.9	49.8†
University degree (%)	21.5	28.6	33.2	38.1†	25.2	33.8	29.1†
Physically active (%)	22.0	24.1	24.2	29.3†	32.7	25.6	24.2†
BMI ≥ 25 kg/m^2 (%)	57.8	56.9	54.9	53.1†	50.9	51.8	57.6†
Current smokers (%)	28.8	22.1	20.9	21.5†	23.9	22.1	23.9†
Hypertension at recruitment [3] (%)	29.2	29.1	27.8	28.2	26.7	28.9	28.6
Hyperlipidemia at recruitment [3] (%)	35.6	36.7	34.5	35.0	38.8	37.0	34.6†
Diabetes mellitus at recruitment [3] (%)	3.1	3.5	3.4	4.8†	3.5	3.5	3.8
Use of anti-hypertensive drugs (%)	13.6	14.7	13.5	14.3	13.8	16.3	13.0†
Use of lipid-lowering drugs (%)	3.3	3.7	4.0	3.9	4.9	4.4	3.3†
Use of NSAIDs (%)	6.0	5.5	5.7	6.5	9.6	8.0	4.8†
Use of calcium supplements (%)	3.1	3.2	3.5	4.4†			

BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition; NSAIDS, non-steroidal anti-inflammatory drugs.

† $P < 0.05$. Values are either percentages or means

[1] Adjusted for total energy intake using the residual method. The cut-off points were 603, 748, and 924 mg/day for men, and 610, 738, and 898 mg/day for women.

[2] Participants with a diagnosis of MI, stroke, or transient ischaemic attack at recruitment and participants whose total energy intake fell into the top or bottom 0.5 sex-specific percentile (men: < 887 / > 5,582 kcal/day; women: < 703 / > 4,381 kcal/day) were excluded. .

[3] Self-reported.

Table 2. Multivariate HRs and 95% CIs for MI and stroke incidence and CVD mortality by quartile of total dietary calcium intake, the EPIC-Heidelberg cohort, 1994 - 2010

	Quartile	No. of cases	HR and 95% CI	
			Model A	Model B
MI incidence	1 (low)	101	1.00 (ref)	1.00 (ref)
	2	91	0.94 (0.70-1.25)	1.01 (0.75-1.37)
	3	70	0.69 (0.50-0.94)	0.67 (0.48-0.94)
	4 (high)	92	0.85 (0.63-1.16)	0.92 (0.66-1.27)
	P_{trend}		0.22	0.39
Stroke incidence	1 (low)	58	1.00 (ref)	1.00 (ref)
	2	79	1.50 (1.06-2.11)	1.38 (0.94-2.01)
	3	64	1.24 (0.86-1.79)	1.28 (0.86-1.89)
	4 (high)	59	1.12 (0.76-1.65)	1.17 (0.77-1.77)
	P_{trend}		0.97	0.73
CVD mortality	1 (low)	65	1.00 (ref)	1.00 (ref)
	2	75	1.34 (0.95-1.88)	1.51 (1.05-2.17)
	3	61	1.15 (0.80-1.65)	1.22 (0.83-1.81)
	4 (high)	66	1.18 (0.82-1.72)	1.30 (0.87-1.94)
	P_{trend}		0.62	0.44

BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; EPIC, European Investigation into Cancer and Nutrition, HR, hazard ratio; MI, myocardial infarction.
Model A: adjusted for sex, age at recruitment, educational level, physical activity, BMI, smoking categories, lifetime alcohol intake, energy-adjusted dietary vitamin D, saturated fatty acid, and total protein intake, total energy intake, self-reported diabetes mellitus at recruitment, and use of calcium supplements.
Model B: cardiovascular events that occurred in the first 2 years of follow-up were excluded.

Table 3. Multivariate HRs and 95% CIs for MI and stroke incidence and CVD mortality by quartile of dairy and non-dairy calcium intake, the EPIC-Heidelberg cohort, 1994 - 2010

	Quartile [1]	No. of cases	HR and 95% CI	
			Model A	Model B
Dairy calcium				
MI incidence	1 (low)	104	1.00 (ref)	1.00 (ref)
	2	91	0.91 (0.68-1.21)	0.89 (0.66-1.20)
	3	71	0.68 (0.50-0.93)	0.64 (0.46-0.90)
	4 (high)	88	0.77 (0.57-1.05)	0.77 (0.56-1.07)
	P_{trend}		0.07	0.10
Stroke incidence	1 (low)	71	1.00 (ref)	1.00 (ref)
	2	66	1.01 (0.72-1.42)	0.97 (0.67-1.40)
	3	58	0.91 (0.64-1.31)	0.88 (0.60-1.31)
	4 (high)	65	1.01 (0.70-1.47)	1.04 (0.70-1.55)
	P_{trend}		0.91	0.90
CVD mortality	1 (low)	70	1.00 (ref)	1.00 (ref)
	2	65	1.07 (0.76-1.50)	1.17 (0.81-1.69)
	3	63	1.07 (0.76-1.53)	1.15 (0.78-1.67)
	4 (high)	69	1.09 (0.76-1.58)	1.16 (0.78-1.72)
	Ptrend		0.70	0.56
Non-dairy calcium				
MI incidence	1 (low)	98	1.00 (ref)	1.00 (ref)
	2	84	0.93 (0.69-1.25)	0.94 (0.68-1.28)
	3	73	0.85 (0.62-1.16)	0.82 (0.59-1.15)
	4 (high)	99	1.15 (0.86-1.55)	1.22 (0.90-1.67)
	P_{trend}		0.40	0.24
Stroke incidence	1 (low)	68	1.00 (ref)	1.00 (ref)
	2	53	0.84 (0.58-1.20)	0.91 (0.62-1.35)
	3	61	0.99 (0.70-1.42)	1.01 (0.68-1.49)
	4 (high)	78	1.26 (0.89-1.77)	1.35 (0.93-1.95)
	P_{trend}		0.08	0.07
CVD mortality	1 (low)	87	1.00 (ref)	1.00 (ref)
	2	59	0.77 (0.55-1.08)	0.73 (0.51-1.05)
	3	58	0.80 (0.57-1.13)	0.82 (0.57-1.17)
	4 (high)	63	0.89 (0.63-1.25)	0.81 (0.56-1.17)
	P_{trend}		0.53	0.32

BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; EPIC, European Investigation into Cancer and Nutrition, HR, hazard ratio; MI, myocardial infarction.

Model A: adjusted for sex, age at recruitment, educational level, hysical activity, BMI, smoking categories, lifetime alcohol intake, energy-adjusted dietary vitamin D, saturated fatty acid, and total protein intake, total energy intake, self-reported diabetes mellitus at recruitment, and use of calcium supplements. Dairy calcium intake and non-dairy calcium intake were also mutually adjusted for.

Model B: cardiovascular events that occurred in the first 2 years of follow-up were excluded.

[1] For dairy calcium intake, the cut-off points were 236, 372, and 552 mg/day for men and 283, 410, and 572 mg/day for women. For non-dairy calcium intake, the cut-off points were 319, 359, and 404 mg/day for men and 280, 316, and 357 mg/day for women.

Table 4. Multivariate HRs and 95% CIs for MI and stroke incidence and CVD mortality by calcium supplementation status, the EPIC-Heidelberg cohort, 1994 - 2010

	Supplements	No. of cases	HR and 95% CI			
			Model A	Model B	Model C	Model D
MI incidence						
	Non-use of any supplements	256	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Calcium	20	1.86 (1.17-2.96)	1.79 (1.09-2.96)	1.28 (0.89, 1.85)	1.20 (0.93, 1.54)
	Calcium only [1]	7	2.39 (1.12-5.12)	2.70 (1.26-5.79)	2.17 (1.06, 4.42)	1.44 (0.80, 2.61)
	Calcium plus others [2]	13	1.66 (0.95-2.93)	1.45 (0.77-2.75)	1.14 (0.76, 1.72)	1.16 (0.88, 1.52)
	Other supplements	78	0.75 (0.58-0.97)	0.75 (0.57-0.99)	0.86 (0.67, 1.12)	0.86 (0.72, 1.01)
Stroke incidence						
	Non-use of any supplements	179	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Calcium	10	1.05 (0.55-1.99)	1.04 (0.51-2.14)	1.08 (0.69, 1.68)	0.93 (0.66, 1.30)
	Calcium only [1]	1	0.34 (0.05-2.47)	0.43 (0.06-3.08)	0.59 (0.15, 2.41)	0.55 (0.18, 1.68)
	Calcium plus others [2]	9	1.35 (0.69-2.66)	1.31 (0.61-2.82)	1.17 (0.74, 1.86)	1.00 (0.70, 1.43)
	Others	71	0.87 (0.66-1.16)	0.97 (0.71-1.31)	0.86 (0.64, 1.15)	0.89 (0.74, 1.09)
CVD mortality						
	Non-use of any supplements	184	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Calcium	9	1.02 (0.51-2.00)	1.04 (0.51-2.15)	1.14 (0.76, 1.72)	1.07 (0.82, 1.41)
	Calcium only [1]	3	1.20 (0.38-3.78)	1.39 (0.44-4.39)	1.54 (0.63, 3.78)	1.23 (0.63, 2.40)
	Calcium plus others [2]	6	0.94 (0.41-2.15)	0.91 (0.37-2.24)	1.08 (0.70, 1.69)	1.05 (0.78, 1.41)
	Other supplements	74	0.94 (0.71-1.24)	0.92 (0.68-1.24)	0.96 (0.72, 1.28)	0.88 (0.74, 1.05)

BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; EPIC, European Prospective Investigation into Cancer and Nutrition; HR, hazard ratio; MI, myocardial infarction; NSAIDS, non-steroidal anti-inflammatory drugs.
Model A: adjusted for sex, age at recruitment, educational level, physical activity, BMI, smoking categories, lifetime alcohol intake, energy-adjusted total dietary calcium, vitamin D, saturated fatty acid, and total protein intake, total energy intake, and self-reported hyperlipidemia and diabetes mellitus at recruitment, and use of NSAIDs.
Model B: excluded cardiovascular events that occurred in the first 2 years of follow-up.
Model C: the effects of the most recent supplementation.
Model D: the effects of the cumulative supplementation. The HRs indicate the relative risks for each self-report of supplementation
[1] $n = 256$.
[2] $n = 695$.